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Soluble Epoxide Hydrolase Gene Deletion Is Protective Against Experimental Cerebral Ischemia

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Abstract

Background and Purpose—Cytochrome P450 epoxygenase metabolizes arachidonic acid to epoxyeicosatrienoic acids (EETs). EETs are produced in the brain and perform important biological functions, including vasodilation and neuroprotection. However, EETs are rapidly metabolized via soluble epoxide hydrolase (sEH) to dihydroxyeicosatrienoic acids (DHETs). We tested the hypothesis that sEH gene deletion is protective against focal cerebral ischemia through enhanced collateral blood flow.

Methods—sEH knockout (sEHKO) mice with and without EETs antagonist 14, 15 epoxyeicosa-5 (Z)-enoic acid (EEZE) were subjected to 2-hour middle cerebral artery occlusion (MCAO), and infarct size was measured at 24 hours of reperfusion and compared to wild-type (WT) mice. Local CBF rates were measured at the end of MCAO using iodoantipyrine (IAP) autoradiography, sEH protein was analyzed by Western blot and immunohistochemistry, and hydrolase activity and levels of EETs/DHETs were measured in brain and plasma using LC-MS/MS and ELISA, respectively.

Results—sEH immunoreactivity was detected in WT, but not sEHKO mouse brain, and was localized to vascular and nonvascular cells. 14,15-DHET was abundantly present in WT, but virtually absent in sEHKO mouse plasma. However, hydrolase activity and free 14,15-EET in brain tissue were not different between WT and sEHKO mice. Infarct size was significantly smaller, whereas regional cerebral blood flow rates were significantly higher in sEHKO compared to WT mice. Infarct size reduction was recapitulated by 14,15-EET infusion. However, 14,15-EEZE did not alter infarct size in sEHKO mice.

Conclusions—sEH gene deletion is protective against ischemic stroke by a vascular mechanism linked to reduced hydration of circulating EETs.

Keywords

stroke; cerebral ischemia; EETs; EEZE, P450 epoxygenase; eicosanoids; neuroprotection; CBF; sEH; EPHX2

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Disclosures

None.

Arachidonic acid (AA) is metabolized to biologically active products collectively referred to as eicosanoids. Three major pathways have been identified for eicosanoid formation in brain: the cyclooxygenase, lipoxygenase and cytochrome P450 pathways.¹ The P450 epoxygenase pathway metabolizes AA to epoxyeicosatrienoic acids (EETs). Depending on which of 4 double bonds in the chemical structure of AA is replaced by an epoxide group, 4 regio-isomers of EETs can be generated: 5,6-EET; 8,9-EET; 11,12-EET and, the focus of this study, 14,15-EET. In the brain, EETs are produced by astrocytes^{2,3} and play an important role in cerebral blood flow (CBF) regulation⁴ and neurovascular coupling.⁵ Furthermore, the expression of P450 epoxygenase in brain is increased by ischemic preconditioning, which was associated with protection from ischemic stroke induced in the rat by middle cerebral artery occlusion (MCAO).⁶ More recently, we demonstrated that EETs protect neurons⁷ and astrocytes⁸ against ischemic cell death induced in vitro by oxygen-glucose deprivation (OGD), suggesting that EETs may exert a cytoprotective effect independent of its effect to dilate blood vessels and increase CBF.

The biological activity of EETs in vivo is limited by their metabolic conversion through multiple pathways.⁹ A major enzyme involved in the metabolism of EETs is soluble epoxide hydrolase (sEH) which preferentially metabolizes 14,15-EET to 14,15-dihydroxyeicosatrienoic acid (14,15-DHET).¹⁰ Indeed, pharmacological inhibition of sEH attenuates OGD-induced cell death in neuronal culture⁷ and reduces infarct size after MCAO in mice.¹¹ In the current study, we follow-up on studies using sEH pharmacological inhibitors by subjecting mice with targeted deletion of sEH to MCAO, and comparing infarct size, sEH protein expression, EETs and DHET levels and regional CBF in these mice to wild-type (WT) control mice. Our findings suggest that deletion of sEH is protective against ischemic brain injury, and that protection is linked to reduced vascular hydrolase activity, reduced circulating 14,15-DHET and enhanced tissue perfusion during MCAO.

Materials and Methods

The study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research, and protocols were approved by animal care and use committee of Oregon Health and Science University, Portland, Oregon.

Animals

Mice with targeted deletion of soluble epoxide hydrolase (sEH knockout, sEHKO)¹² were obtained from Dr Frank Gonzalez at the National Cancer Institute. The mice originated on a B6; 129X1 background and have been backcrossed to C57BL/6 for at least 6 generations, and therefore, homozygous sEHKO mice were compared to WT C57BL/6 mice obtained from The Jackson Laboratories. Homozygous mice are viable, fertile, normal in size and do not display any gross physical or behavioral abnormalities. Mice were genotyped by PCR as previously described.¹²

MCAO in Mice

Transient focal cerebral ischemia was induced in adult male mice (20 to 26 g body weight) using the intraluminal MCAO technique, as previously described.¹³ Briefly, mice were anesthetized with halothane (1.5 to 2% in O₂-enriched air by face mask), and kept warm with water pads. A small laser-Doppler probe was affixed to skull to monitor cortical perfusion and verify vascular occlusion and reperfusion. A silicone-coated 6–0 nylon monofilament was inserted into the right internal carotid artery (ICA) via the external carotid artery (ECA) until laser-Doppler signal dropped to <30% of baseline. After securing the filament in place, the surgical site was closed, and the animal was awakened and assessed at 2 hours of occlusion for neurological deficit using a simple neurological scoring system as follows: 0=no deficit,

1=failure to extend forelimb, 2=circling, 3=unilateral weakness, 4=no spontaneous motor activity. Mice with neurological deficit score between 1 to 3 were reanesthetized, laser-Doppler probe repositioned over same site on the skull, and the occluding filament was withdrawn to allow for reperfusion. Mice were then allowed to recover and were observed for 1 day. Infarct size was measured at 24 hours after MCAO in 2-mm thick coronal brain sections (5 total) using 2,3,5-triphenyltetrazolium chloride (TTC) staining and digital image analysis. Sections were incubated in 1.2% TTC in saline for 15 minutes at 37°C, and then fixed in formalin for 24 hours. Slices were photographed and infarcted (unstained) and uninjured (red color) areas were measured with MCID software (InterFocus) and integrated across all 5 slices. To account for the effect of edema, infarct size was estimated indirectly by subtracting the uninjured area in the ipsilateral hemisphere from the contralateral hemisphere, and expressing infarct volume as a percentage of the contralateral hemisphere. Separate groups of nonsurvival WT and sEHKO mice (n=5 per group) were kept under anesthesia for 2 hours to continuously monitor laser-Doppler cere-brocortical tissue perfusion and arterial blood pressure and gases. To determine whether 14,15-EET is protective against ischemic brain injury, we administered synthetic 14,15-EET (1 µg) over 24 hours via jugular vein catheter connected to a subcutaneously implanted osmotic minipump. To determine whether protection in sEHKO mice was mediated via 14,15-EET, we examined the effect of 14,15-EET antagonist 14, 15 epoxyeicosa-5(Z)-enoic acid 14,15-EEZE¹⁴ (10 µg over 24 hours via minipump implanted 1 hour before MCAO).

Western Blot and Immunohistochemistry

Brain tissue from WT versus sEHKO mice were homogenized in solution A containing sucrose (250 mmol/L), KCl (60 mmol/L), TrisHCl (15 mmol/L), NaCl (15 mmol/L), EDTA (5 mmol/L), EGTA (1 mmol/L), PMSF (0.5 mmol/L), and DTT 10 mmol/L), then centrifuged at 2000g for 10 minutes at 4°C. Protein samples (30 µg) were separated by SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes. Blots were then blocked in 5% dry milk and incubated at 4°C overnight with a primary rabbit anti-sEH antibody.^{11,15,16} Signal was visualized using a biotinylated secondary antibody (Amersham) with an ECL plus chemiluminescence detection kit (Amersham). Autoradiograms were scanned and band optical densities quantified with QuantityOne software (BioRad). Blots were reprobed for β-actin (Sigma) to ensure equal loading. To localize sEH immunoreactivity in brain, thin coronal sections were probed with anti-sEH antibody, as previously described.¹¹

Atmospheric Pressure Chemical Ionization (APCI) Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

The amount of free 14,15-EET from brain tissue of WT versus sEHKO mice was determined using electron capture APCI LC-MS/MS of the pentafluorobenzyl-esters (PFB) of the EETs.¹⁷ We adopted the extraction method of Yue et al.¹⁸ Briefly, frozen brain tissue (approximately 50 mg) was added to 200 µL methanol containing 0.2% formic acid and 1000 pg (±) 14,15-EET-d₈ (BioMol Research Laboratories) internal standard was added. The tissue was homogenized on ice with a microultrasonic cell disruptor at a setting of 6.5 (2 mm probe, highest setting 20, Microscan Ultrasonic). Following centrifugation at 14 000 rpm for 15 minutes, the supernatants were diluted with water to a final methanol concentration of 10% and loaded onto a 1 mL Oasis HLB SPE cartridge preconditioned as described.¹⁸ The cartridges were washed with 1 mL of water and 10% methanol, and dried under nitrogen for 30 minutes. The metabolites were eluted with anhydrous acetonitrile and dried under nitrogen. Dried extracts were dissolved in 100 µL of anhydrous acetonitrile and derivatized at 60°C after the addition of 100 µL of 2.5% 2,3,4,5,6-pentafluorobenzyl (PFB) bromide (99% Aldrich) in anhydrous acetonitrile and 5% N,N-diisopropylethylamine (redistilled, 99.5% Aldrich) in anhydrous acetonitrile.¹⁷ The reaction was cooled to room temperature and the PFB-esters were extracted with hexane after the addition of equal volume of 0.15 mol/L KCl solution. The

hexane extract was dried and the residue dissolved in 50 μ L of 85% methanol for analysis by LC-MS/MS. EET standards were obtained from Cayman. The PFB-esters of DHETS, HETEs and EETs were resolved using a Surveyor HPLC system with a 50 \times 2.1 mm (5 μ m) Betabasic C18 HPLC column (ThermoHypersil) at a flow rate of 0.3 mL/min with a gradient from 75% to 98% methanol over 35 minutes. Mass spectrometry was conducted with a TSQ Quantum Discovery triple-quadrupole mass spectrometer equipped with an APCI source in negative mode. The instrument operating conditions were as follows: vaporizer temperature, 350°C; capillary temperature, 200°C; capillary offset, -35 V; corona discharge needle, set at 18 μ A. The sheath and auxiliary gas were 35 psi and 2 arbitrary units, respectively. Collision induced dissociation was performed with argon as the collision gas at 0.8 mTorr in the second quadrupole. Unit resolution was maintained for precursor and products ions. 14,15-EET was monitored in the selected reaction monitoring mode with transitions of $m/z=319\rightarrow175$ as well as $m/z=319\rightarrow219$. The deuterated internal standard, 14,15-EET- d_8 , was monitored with the transition of $m/z=327\rightarrow226$. The amount of 14,15-EET was calculated by comparison of the area ratio of 14,15-EET to 14,15-EET- d_8 to those obtained when brain tissue was spiked with known amounts of 14,15-EET.

14,15-DHET ELISA

Total 14,15-DHET in blood was measured with a commercial ELISA (Detroit R&D, Inc, Detroit, Mich).⁷ Briefly, plasma lipids were extracted 3 times with ethyl acetate under acidic conditions (pH 3 to 4), followed by saponification with 20% KOH. Samples were dried and resuspended in methanol, and 14,15-DHET concentration was measured according to the manufacturer's instructions. The ELISA was also used to measure hydrolase activity in brain tissue (perfused to remove blood) incubated with 1 μ M 14,15-EET for 30 minutes. Brain tissue was homogenized in 4 volumes of ice-cold buffer (20 mmol/L Tris HCL pH 7.4, 0.32 mol/L sucrose, 1 mmol/L EDTA), centrifuged at 1000g for 10 minutes, and supernatant was further centrifuged at 10 000g for 20 minutes.

Regional CBF Measurement Using [¹⁴C]-Iodoantipyrine (IAP) Autoradiography

End-ischemic regional CBF was measured in a nonsurvival cohort of sEHKO or C57Blk/6 mice using quantitative autoradiography with [¹⁴C] IAP, as previously described.¹¹ Mice were instrumented with femoral artery and jugular vein catheters, and MCA occluded as in the previous cohort. At 2 hours of MCA occlusion, 1 μ Ci of [¹⁴C] IAP in 75 μ L of saline was infused intravenously for 45 seconds. Free-flowing arterial blood was simultaneously sampled at 5-second intervals for the arterial input function. With the filament in place, mice were decapitated at 45 seconds after the start of infusion, and the brain was quickly removed and frozen in 2-methylbutane on dry ice. Brains were sectioned on a cryostat into 20- μ m-thick coronal slices, which were thaw-mounted on cover glass and then apposed for 3 weeks to film (Kodak, Bio-Max) together with ¹⁴C standards. The concentration of [¹⁴C] IAP in blood was determined by liquid scintillation spectroscopy (model 6500, Beckman) after decolorization with 0.2 mL of tissue solubilizer (Soluene-350, PerkinElmer, Inc). Autoradiographic images representing 5 different coronal levels (+2, +1, 0, -1 and -2 from Bregma, 3 images each) were digitized, and rCBF was determined in specific regions with the use of image analysis software (MCID, 7.0). Additionally, areas with similar flow rates were isolated and summed to construct a histogram of brain tissue volumes perfused with given blood flow rates.

Statistical Analysis

Differences in infarct size, DHET levels and CBF rates were analyzed with a *t* test for 2 groups and analysis of variance (ANOVA) with post hoc Newman-Keuls multiple range test for multiple groups. The criterion for statistical significance is $P<0.05$. All values are reported as mean \pm SEM.

Results

Figure 1 illustrates PCR-based genotyping strategy of WT (+/+, 233 bp) and sEHKO mice (-/-, 190 bp) (A), and demonstrates that sEH protein is abundantly expressed in WT, but not in homozygous sEHKO mouse brain (62.5 kDa, n=4) (B). Figure 1 C shows representative LC-MS/MS extracted ion chromatograms (XICs) depicting free endogenous brain 14,15-EET peaks in WT and sEHKO mouse brains. The levels of free 14,15-EET detected in brain tissue were not statistically different between WT and sEHKO mice: 483 pg per 50 mg tissue (\pm SD 93 pg, n=3) compared to 735 pg (\pm SD 382 pg, n=3), respectively. Similarly, there were no differences in brain tissue levels of other EETs regioisomers between WT and sEHKO mice. Furthermore, there were no differences in brain hydrolase activities between WT and sEHKO mice, as indicated by the ability of brain tissue from these mice to convert 14,15-EET to corresponding DHET *ex vivo*. Interestingly, the metabolism of 14,15-EET to 14,15-DHET in both WT and KO brain tissue was inhibited by the epoxide hydrolase inhibitor AUDA-BE (data not shown).¹¹ Finally, we measured levels of total 14,15-DHET in WT and sEHKO mouse plasma using ELISA.⁷ Total 14,15-DHET in plasma was significantly lower in sEHKO than WT mice (115 \pm 73, n=10, versus 4179 \pm 482 pg/mL, n=12, P <0.001). Figure 2 demonstrates that sEHKO mice sustained smaller infarct after MCAO compared to WT mice (16 \pm 6% compared to 36 \pm 3%, respectively, n=5 per group, P <0.05). Neurological deficit during and at 24 hours after MCAO was significantly smaller in sEHKO compared to WT mice (data not shown). Figure 3A illustrates that exogenous 14,15-EET significantly reduces infarct size after MCAO in mice from 34.5 \pm 8.6% to 10.9 \pm 4.3% (n=4 per group, P <0.05). However, 14,15-EEZE administration did not alter infarct size (9.4 \pm 12.4 [mean \pm SD, n=9]) compared to vehicle-treated sEHKO mice (9.1 \pm 9.5, n=5, P =0.97). Laser-Doppler monitoring in surviving WT and sEHKO mice suggested that tissue perfusion was reduced to the same degree in both groups. However, continuous monitoring of laser-Doppler perfusion for the entire 2-hour MCAO period revealed that laser-Doppler perfusion remained stably low in WT mice, whereas tissue perfusion slowly increased in sEHKO mice, and by 30 minutes was significantly higher than WT mice (Figure 3B). To confirm that blood flow rates were higher in sEHKO versus WT mice at the end of MCAO, we used [¹⁴C]iodoan-tipyrine (IAP) autoradiography to determine absolute regional CBF rates in WT and sEHKO mouse brain at the end of 2-hour MCAO. Figure 4A is color-coded distribution of CBF in ischemic and contralateral hemispheres, demonstrating higher tissue perfusion in ipsilateral hemisphere at end ischemia in sEHKO compared to WT mice (representative of 5 mice per group). Analysis of blood flow distribution in the two strains revealed that the amount of tissue perfused with ischemic flow rates (0 to 20 mL/100 g/min) were significantly lower in sEHKO compared to WT mice at the end of MCAO (P <0.05, n=5, not shown). Finally, immunohistochemical analysis localized sEH expression in cerebral blood vessels (Figure 4B).

Discussion

We report the following new findings: (1) Targeted deletion of sEH is protective against focal cerebral ischemia, but protection was not diminished by EETs antagonist 14,15-EEZE, (2) Brain tissue perfusion is higher in sEH null compared to WT mice during vascular occlusion, (3) Brain tissue hydrolase activity and levels of free 14,15-EET were not different between sEHKO and WT mice, despite the absence of sEH immunoreactivity in knockout mouse brain, (4) The level of total 14,15-DHET in blood was lower in sEHKO compared to WT mice, and finally (5) Immunoreactivity was detected in cerebral vessels from WT mice. We conclude that sEH gene deletion is protective against ischemic brain injury, in part due to reduced metabolism of circulating 14,15-EET and enhanced vasodilator capacity and collateral blood flow in response to focal vascular occlusion. The findings also suggest that circulating 14,15-EET serves a protective function, and that manipulations aimed at decreasing its metabolism may be protective against ischemic stroke.

The lack of difference in 14,15-EET between WT and sEHKO mice was surprising, and suggested that sEHKO mouse brain may have an alternative mechanism for EETs hydrolysis other than sEH. This enzymatic activity, however, was inhibited by sEH inhibitor AUDA- BE,¹¹ and was present in sEHKO brain but not peripheral tissue. The source of circulating EETs and tissue localization of hydrolase activity responsible for DHET in blood is unclear. The most obvious source for EETs is vascular endothelium and the most obvious location of sEH is vascular smooth muscle, although contributions from liver, kidney, lungs and other tissues, including blood cells, cannot be ruled out. In support of a vascular site for EETs hydrolysis, immunohistochemistry localized sEH in cerebral blood vessels. This is consistent with our previous study demonstrating sEH expression in both vascular and nonvascular brain compartments.¹¹ The identity of the residual hydrolase activity in sEHKO brain is unknown. One possibility is that the microsomal epoxide hydrolase (mEH), which can potentially metabolize 14,15-EET, albeit less efficiently than sEH, may assume a more prominent role in EETs metabolism in brain when sEH is deleted. The fact that no such compensation takes place in peripheral tissues may be related to the differential levels of expression or regulation of mEH in neural versus peripheral tissues.

To determine whether 14,15-EET is responsible for the protection in sEHKO mice, we used the 14,15-EET antagonist 14,15-EEZE.¹⁴ However, 14,15-EEZE administration did not increase infarct size in sEHKO mice. This may indicate that protection in sEHKO mice is not mediated through 14,15-EET, or it may be related to the observation that 14,15-EEZE exhibits agonistic activity in some tissues.¹⁹ In our study, we did not measure 14,15-EET levels in blood using LC-MS/MS. However, a recent report has demonstrated using LC-MS/MS that the levels of epoxy fatty acids, including 14,15-EET, were elevated in blood, liver and kidney in two sEHKO mouse lines, including the one used in this study (the “NIH” line), compared to WT mice.²⁰ It should be noted, however, that despite the evidence that protection in sEHKO mice was associated with reduced circulating 14,15-DHET, and that it was recapitulated by exogenous 14,15-EET administration, our data does not completely exclude contributions by other EETs regioisomers. It is also possible that the protection may not at all be related to EETs, since sEH is a bifunctional enzyme, with a C-terminal hydrolase and an N-terminal phosphatase. Nevertheless, data are still extremely limited regarding possible endogenous substrates for the N-terminal phosphatase,²¹ and attempts to elucidate the enzyme’s phosphatase function and relevance to stroke are hampered by the inability of known phosphatase inhibitors to block the N-terminal phosphatase activity of sEH.²²

Our finding here that sEH gene deletion is protective against ischemic brain injury is consistent with our previous finding that pharmacological inhibition of sEH reduces infarct size after MCAO in WT mice,¹¹ and that polymorphisms in the sEH gene that alter the enzyme’s hydrolase activity are linked to neuronal survival after ischemic injury.⁷ This is also consistent with the study by Seubert et al²³ demonstrating improved functional recovery after myocardial ischemia in isolated heart preparation from sEHKO mice. Our finding that 14,15-EET is protective in vivo is also consistent with our previous observation that 14,15-EET is protective against cell death induced in primary cortical neurons⁷ and astrocytes⁸ by oxygen-glucose deprivation (OGD). However, the mechanism of neuroprotection by 14,15-EET in vitro is likely different than its mechanism of action in vivo. Our data using laser-Doppler perfusion and iodoantipyrine autoradiography suggest that the mechanism of protection in vivo may be related to EETs’ vasodilator property. This, however, is in contrast to our own previous observation that pharmacological inhibition of sEH was protective against ischemic stroke by a blood flow-independent mechanism.¹¹ The discrepancy may be related to the fact that sEH is a bifunctional enzyme,²⁴ and that while gene deletion eliminates the enzyme’s lipid phosphatase and epoxide hydrolase functions, the inhibitors were designed to inhibit only the hydrolase function. Another possibility is that the level of 14,15-EET achieved by complete gene deletion, and required for vasodilation, is much higher than that achieved by partial

pharmacological inhibition. It should be noted, however, that EETs possess other properties that can potentially explain the protective effect of sEH deletion. For example, EETs have been shown to suppress inflammation,²⁵ hyperthermia,²⁶ platelet aggregation,²⁷ generation of reactive oxygen species,²⁸ and apoptosis,²⁹ all of which are known to exacerbate ischemic brain injury.

In summary, the current study provides the first characterization of CBF and ischemic brain injury in sEH null mice, and demonstrates that sEH gene deletion is associated with smaller infarct, higher circulating, but not brain 14,15 EET, and higher blood flow during focal vascular occlusion. The findings suggest that vascular sEH may play an important role in regulating EETs' circulating levels, and may serve as a therapeutic target for increased tissue perfusion and protection from ischemic brain injury.

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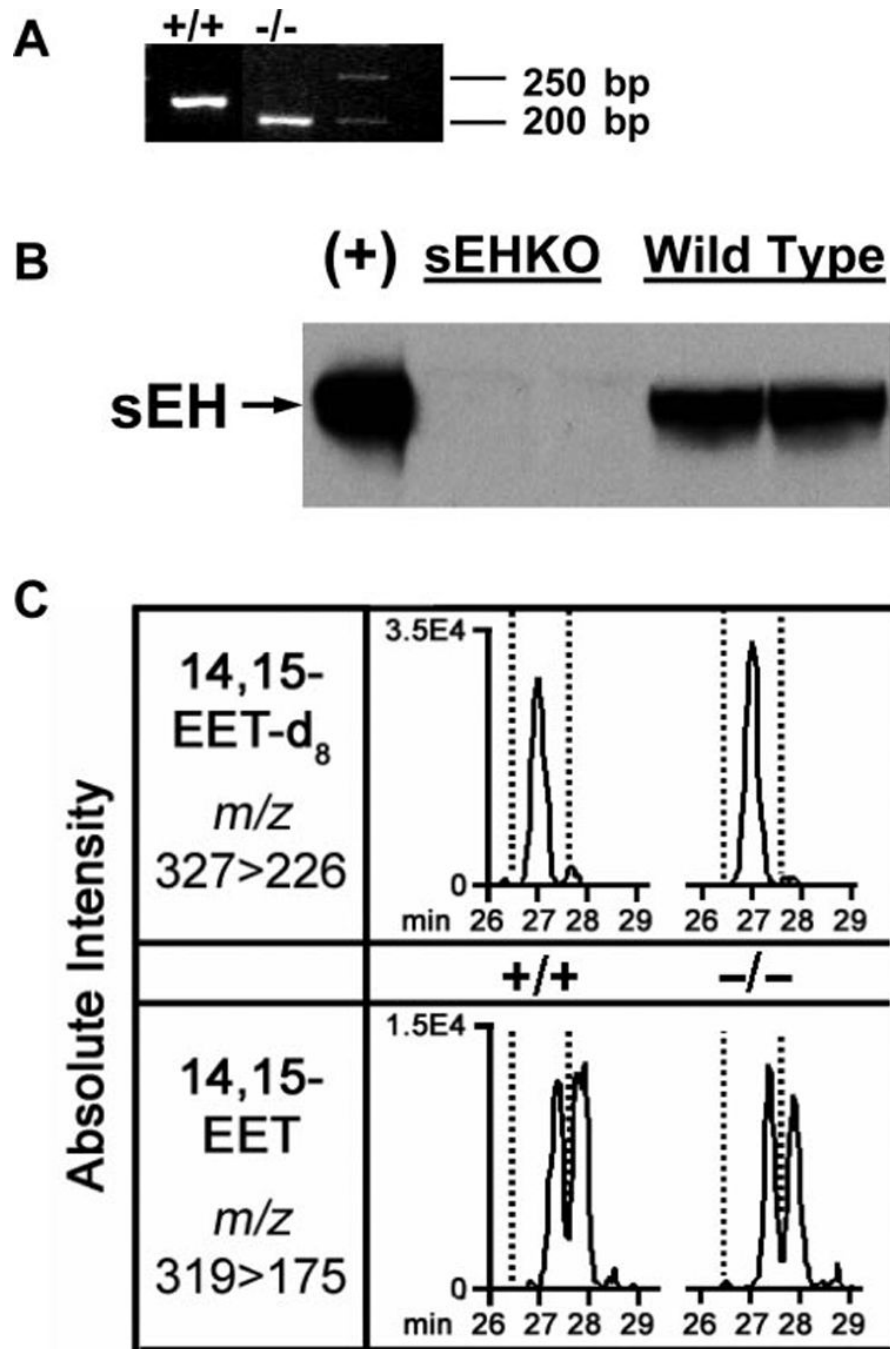


Figure 1. sEH expression and levels of free 14,15-EET in brain

A, PCR genotyping showing deletion of sEH gene in sEHKO mice (-/-, 190 bp) compared to WT mice (+/+, 233 bp). B, Western blot of mouse brain tissue using anti-sEH antibody, demonstrating lack of sEH protein in sEHKO mouse brain and abundant expression in WT mouse brain, (+) is positive control using purified recombinant sEH. C, LC-MS/MS analysis of 14,15-EET in brain extracts from WT and sEHKO mice. The upper panel extracted ion chromatograms (XICs) monitor for 1000 pg 14,15-EET-d₈ spiked into brain extracts, whereas the lower panel XICs monitor for endogenous 14,15-EET peak in sEHKO versus WT mouse brain extracts. Detection of 14,15-EET was carried out with LC-MS/MS monitoring for the transitions from m/z 319 parent ion to m/z 175 and 219 product ions. The first peak detected

at 27.3 minutes with both transitions was 14,15-EET; separation of the 14,15-EET peak was ensured from a closely eluting unknown molecule peak that was also detected. Shown in Figure 1C are the extracted ion chromatograms monitoring for m/z 319>175 from representative sEHKO (-/-) and WT (+/+) brains.

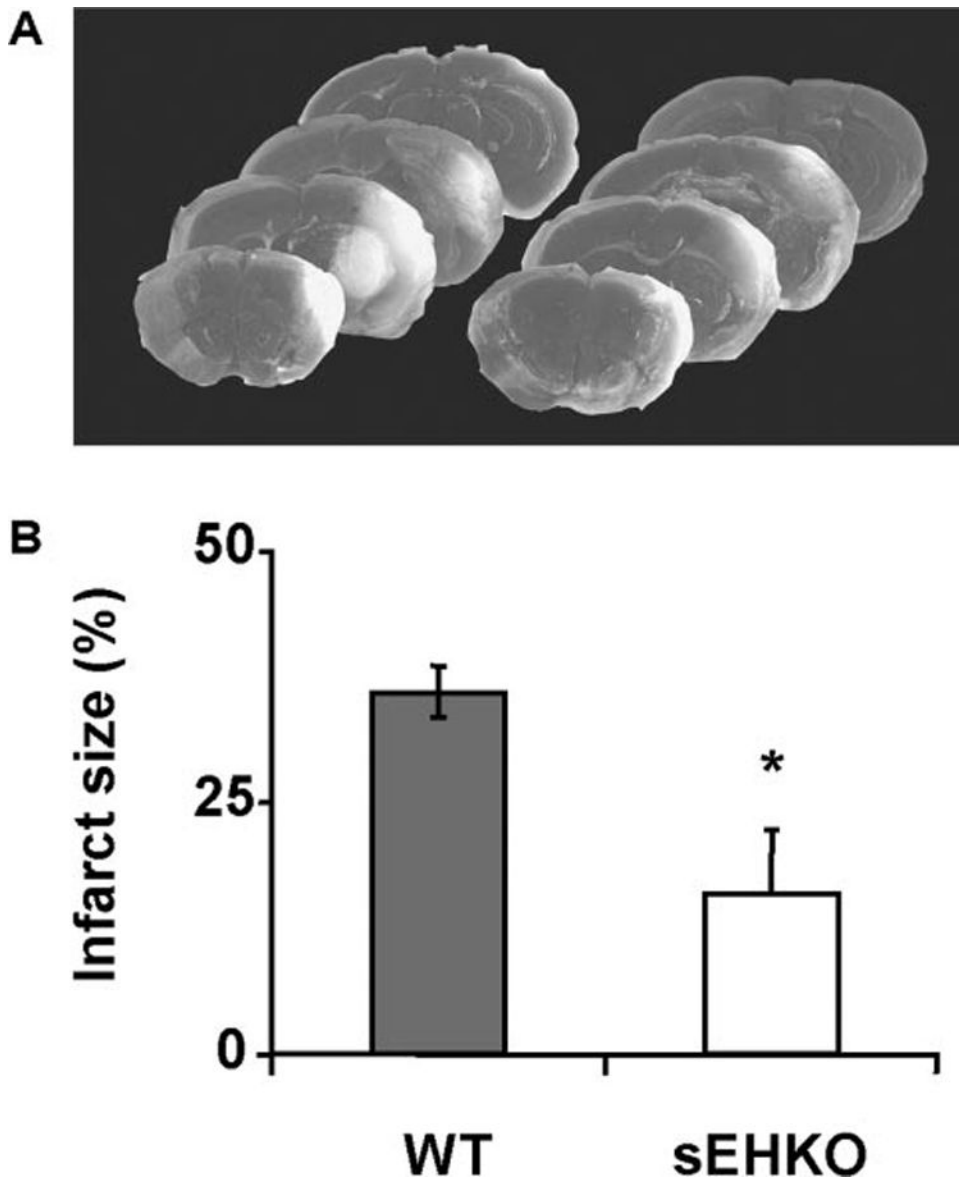


Figure 2. sEHKO mice sustain smaller brain infarcts after MCAO compare to C57Black WT mice
A, Representative TTC-stained brain slices from WT (left) and sEHKO (right) mouse brains.
B, Infarct size was reduced from $36 \pm 3\%$ in WT mice to $16 \pm 6\%$ ($n=5$ per group, $\text{mean} \pm \text{SEM}$) in sEHKO mice. *Different than vehicle ($P < 0.05$).

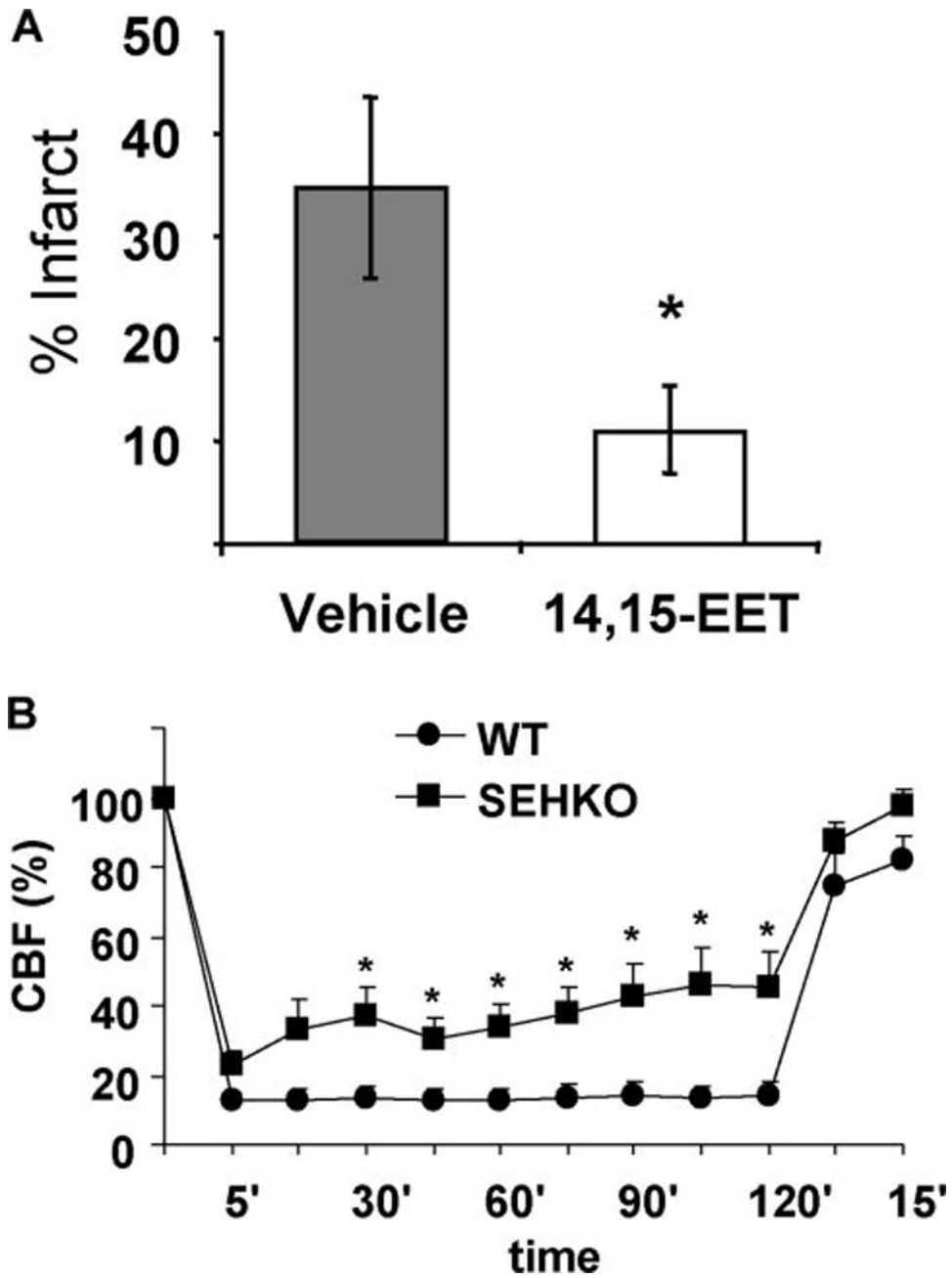


Figure 3. A, Administration of 14,15-EET reduces infarct size after MCAO compared to vehicle-treated mice (n=4 per group, *P<0.05 versus vehicle). 14,15-EET (1 μg) was administered via osmotic minipumps over 24 hours. The pump was implanted subcutaneously 1 hour before MCAO and connected to the jugular vein via catheter, and 14,15-EET was diluted in 10% ethanol in saline. B, Laser-Doppler cerebrocortical tissue perfusion monitoring during MCAO in WT and sEHKO mice (n=5 per group). Laser-Doppler perfusion was decreased in both groups to a similar degree on occlusion. However, while perfusion remained stable during MCAO in WT mice, in sEHKO mice, laser-Doppler slowly recovered and was significantly higher than WT starting at 30 minutes after MCAO onset. *Different than WT (P<0.05).

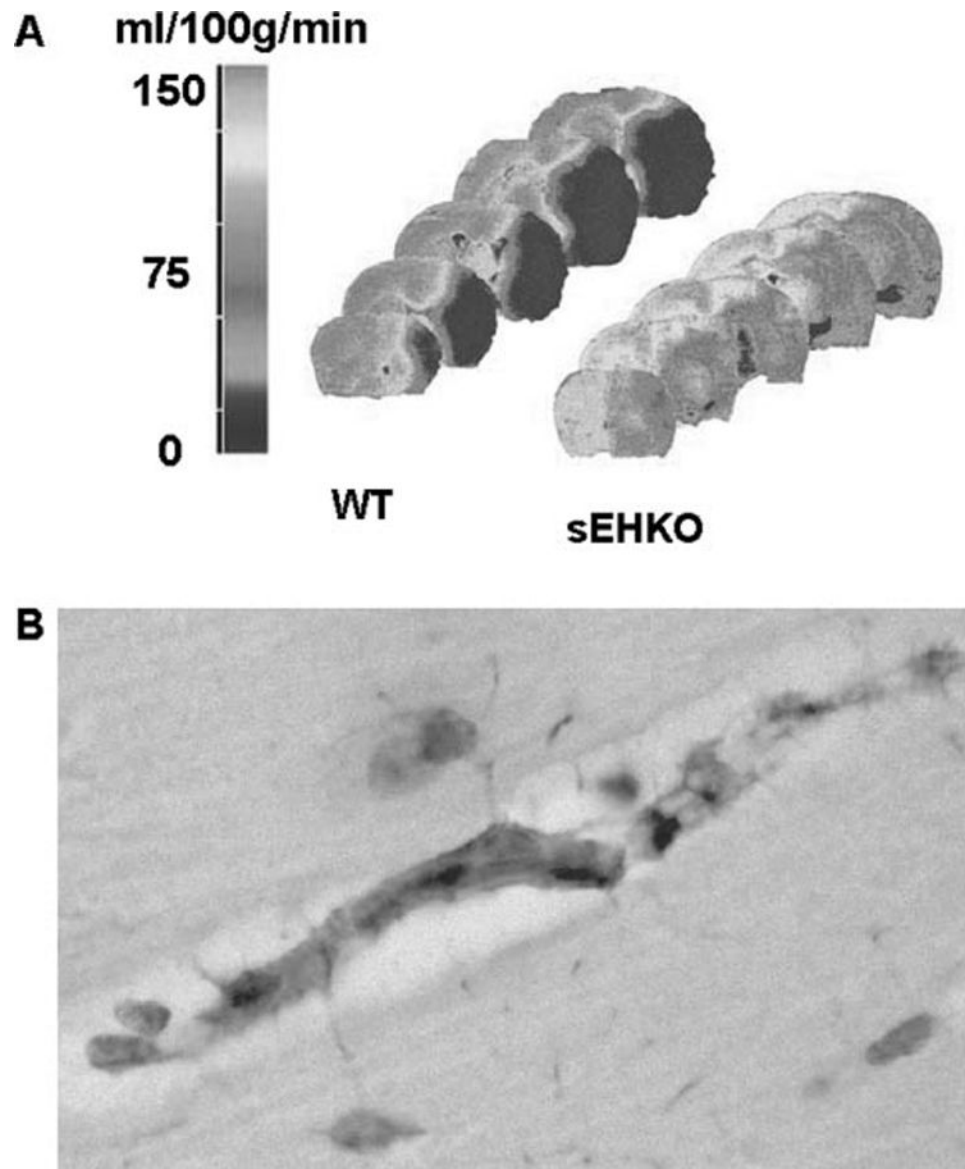


Figure 4. Blood flow rates are higher in sEHKO compared to WT mouse brain during MCAO Flow rates were quantified by [^{14}C]iodoantipyrine (IAP) autoradiography at the end of 2-hour MCAO. A, Color-coded distribution of regional CBF rates in WT (left) and sEHKO (right) mice. B, Immunohistochemical localization of sEH in cerebral blood vessels.